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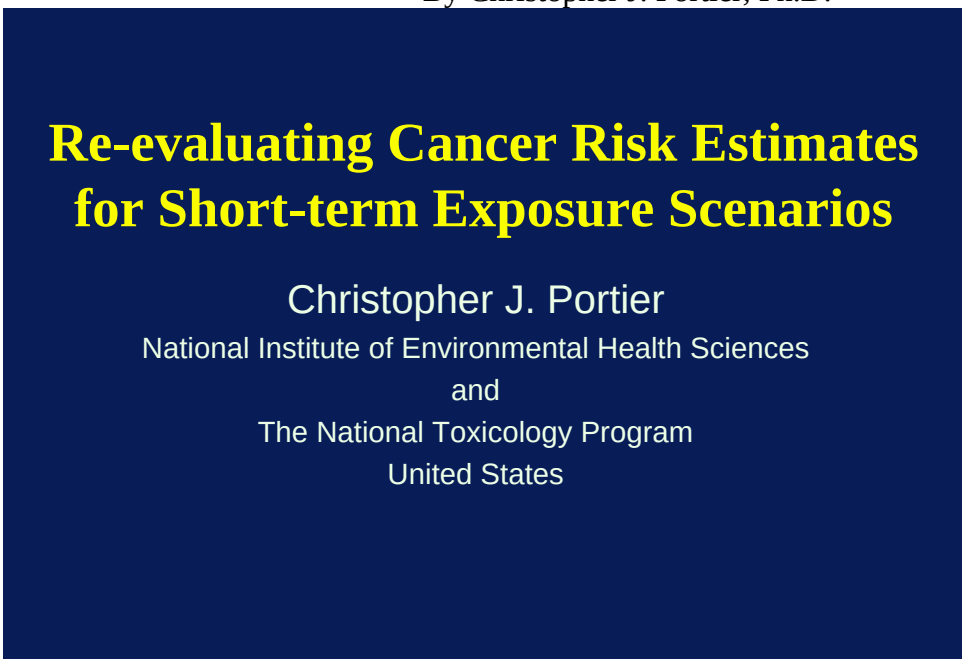
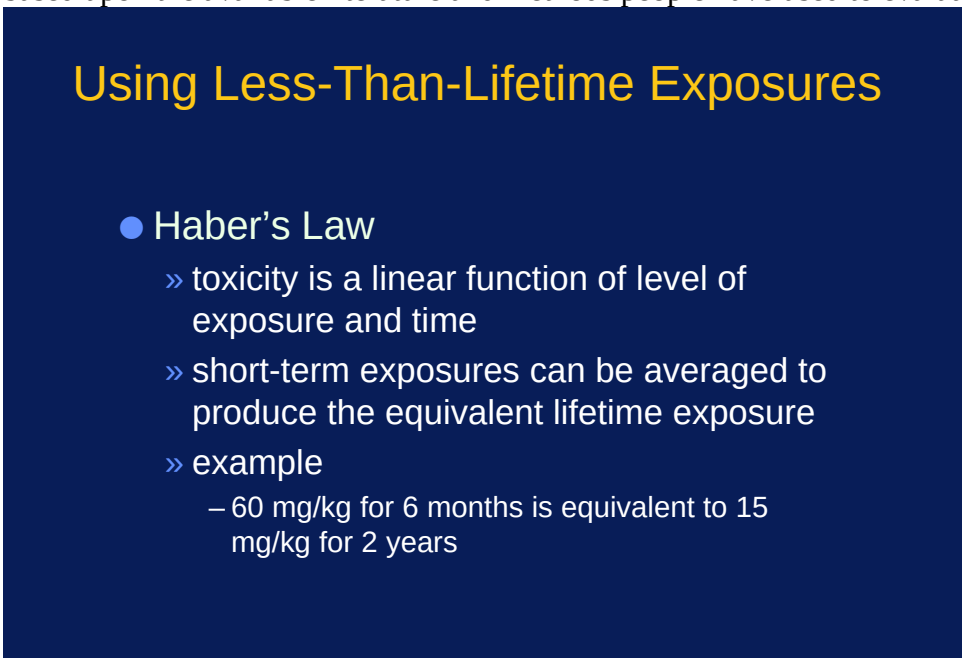
Re-evaluating Cancer Risk Estimates for Short-term Exposure Scenarios

By Christopher J. Portier, Ph.D.

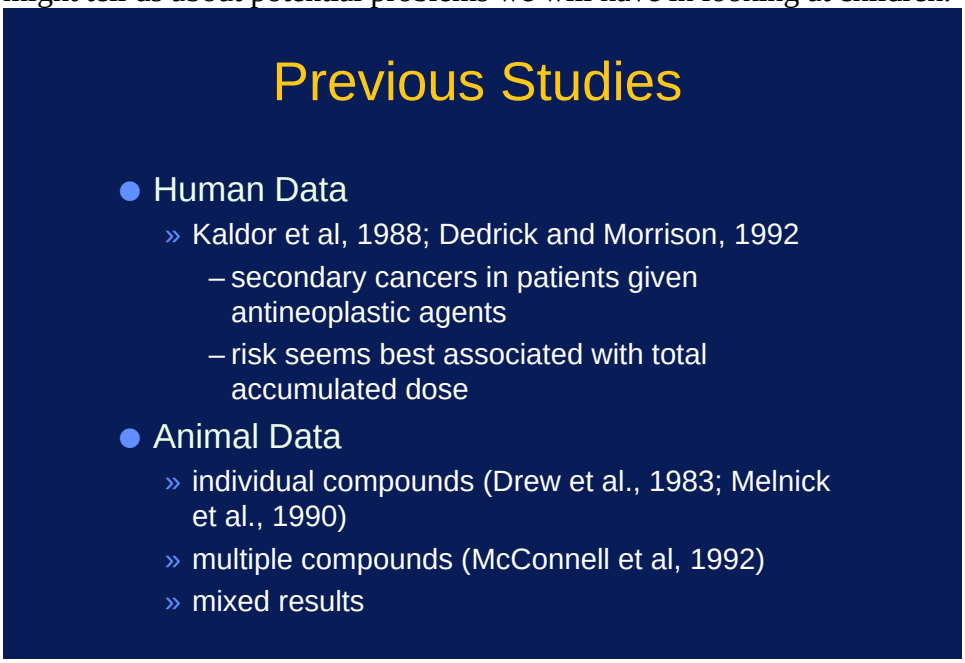


This presentation will look at the issue of cancer risk estimates for short-term exposure scenarios based upon the available literature and methods people have used to evaluate these data.



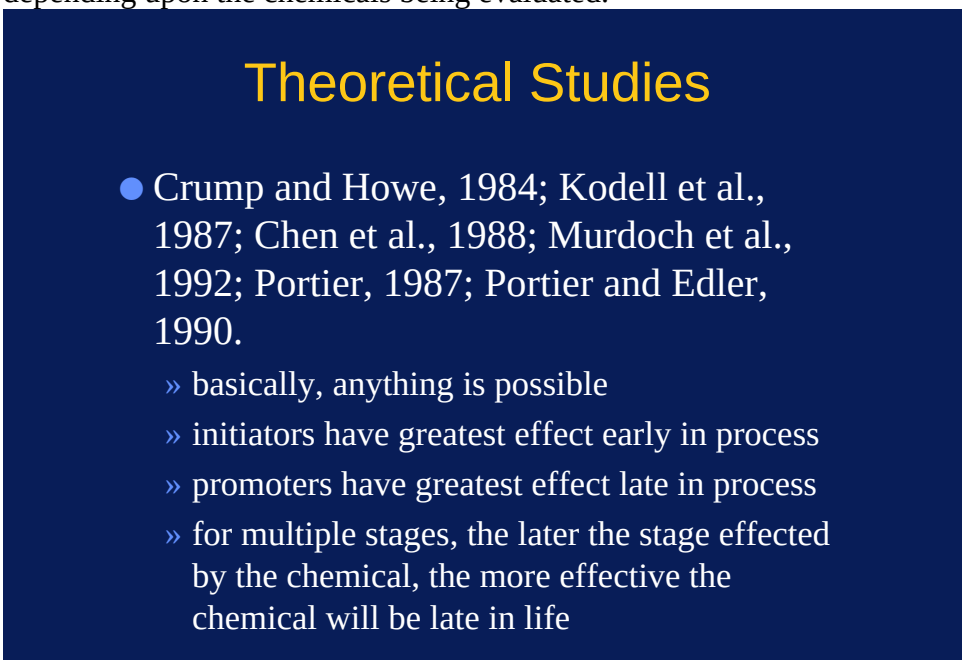
It's common in cancer risk assessment to use what is loosely associated with Haber[1], called Haber's Law, which is based upon acute toxicity associated with gases back in the 1920s and

1930s. Roughly, Haber's Law assumes toxicity is a linear function of the level of exposure and the amount of time that exposure occurs. The general assumption is that short-term exposure can be averaged to produce an equivalent exposure at a lower level over a longer period of time. An obvious example is the one presented here. You can give an animal 60 mg/kg for six months, and this is equivalent to 15 mg/kg for two years. This pertains to children's health in that different windows of exposure are related to completely different patterns of response. What I am going to discuss is not necessarily a childhood issue, per se, because the data that I would have liked to evaluate was not available. So let us look at this issue in adults and see what it might tell us about potential problems we will have in looking at children.



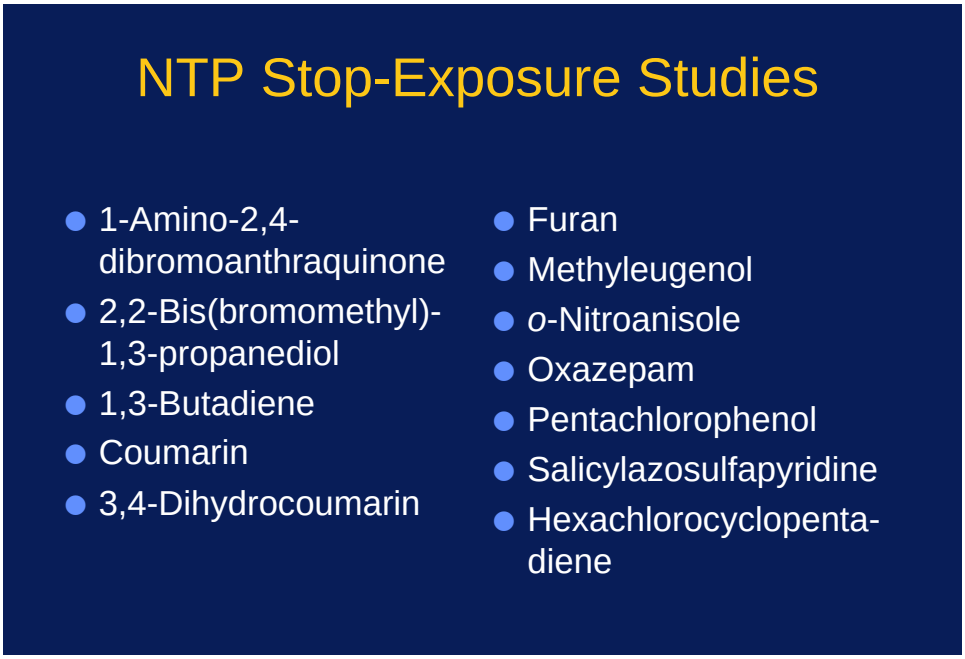
There have been a number of previous studies of this issue in the literature; I am going to cite a few here. I'll begin with the studies by Kaldor[2] and Dedrick and Morrison[3], who studied human populations. Both groups of researchers were looking at secondary cancers that occur in patients given antineoplastic agents. Their basic conclusions from these studies were that risks seem to be best associated with total accumulated dose. So their argument would be that averaging dose is improper in this case; that is, you cannot take an average over a period of time and expect it to get the same answer for short-term and long-term exposures.

In the animal literature, there have been several publications on this and many of them deal with individual compounds. I have given two examples here, one from the National Toxicology Program (NTP), the Drew et al.[4] study, and another NTP study Melnick et al.[5] on separate chemicals. Several groups have looked at multiple compounds. McConnell[6] looked at multiple compounds. A problem with respect to these analyses is that they are not using consistent methods of analyzing the data and they are not evaluating data that are derived in consistent fashions, so that you have analyses that are not easily compared. It is difficult to discuss the implications of these findings across multiple chemical end points as a general rule for risk assessment. Specifically, it is important to identify the response attributes that help us to avoid mistakes in terms of time-averaging of doses. These studies also have multiple and mixed results depending upon the chemicals being evaluated.



Several theoretical studies have looked at this issue as well[7-11]. For example, it is possible to hypothesize a theoretical mechanism by which cancer can arises, and then use this hypothetical mechanism to model what happens to lifetime risk when exposure is early, what happens when exposure is late, what happens if the exposure is short, what happens if it's long, or what happens if it occurs in this narrow window in which important biological events are occurring. There are

a number of studies that have reported these types of analyses. I have done some of these myself, and believe me, I can show you any type of response in these theoretical models. These types of analyses are an informative and insightful means of looking at the question. Much of what we understand about averaging exposure in carcinogenesis studies arise from these types of analyses. The obvious ones deal with initiators and promoters, and the number of stages[11], but there are others as well.



I did not want to walk you through all of these possible mechanisms and mechanistic models; instead, I want to do something slightly different that is solidly linked to data. The National Toxicology Program has done 12 studies[12-23] in which the chronic bioassay includes exposure groups for which the exposure was stopped early in the study but the animals were followed through to the end of the study. These are what I will refer to as the stop-exposure studies of the NTP.

The chemicals studied in these bioassays varied from 1, 3-butadiene[17], which had four separate stop exposures, to some of the others that just had one stop exposure. The 12 compounds studied are shown in the slide (Slide 5).

All of these studies are done under the same good laboratory practice (GLP) protocols. The pathology is conducted under the same methods; the pathological slides are prepared and read the same way. The analyses presented in the technical reports are all identical so we are looking at a fairly consistent dataset to make this comparison. In addition, the data were available for me to make survival adjustments in all of these studies. This is very important since some of these stop-exposure studies have high exposures with some degree of toxicity and you have to adjust for survival differences.

Experimental Designs
NTP Stop-Exposure Studies

Chemical	Use	TR #	Significant Endpoint	Experimental Design [Units] Dose (Weeks on Study)
Methyleugenol	Natural Flavoring	491	Liver, Kidney, Glandular Stomach, Malignant Mesothelioma (clear evidence for each endpoint)	[mg/kg gavage] 0, 37, 75, 150 (105); 300 (52)
o-Nitroanisole	Synthesis of Azo Dyes and *o*-Anisidine	416	Urinary Bladder, Kidney, Large Intestine (clear evidence for each endpoint); Mononuclear Cell Leukemia (chemical-related increase); Forestomach	[ppm feed] 0, 222, 666, 2000 (104); 6000 (27); 18000 (27)
Oxazepam	Anxiolytic Drug	468	Kidney (equivocal evidence)	[ppm feed] 0, 625, 2500, 5000 (up to 105); 10000 (26)

Experimental Designs
NTP Stop-Exposure Studies

Chemical	Use	TR #	Significant Endpoint	Experimental Design [Units] Dose (Weeks on Study)
Pentachlorophenol	Pesticide, Fungicide	483	Nose, Malignant Mesothelioma (some evidence in stop study only)	[ppm feed] 0, 200, 400, 600 (105); 1000 (52)
Salicylazo-sulfapyridine	Anti-microbial Drug	457	Urinary Bladder (some evidence)	[mg/kg gavage] 0, 84, 168, 337.5 (up to 105); 337.5 (26)
Hexachloro-cyclopentadiene		437	No Tumors	

I have listed four separate tables with all of the NTP stop studies. I am not going to walk you through each and every one of them, but I will give you the basics of one of them. Let's look at 2, 2-bis(bromomethyl)-1,3-propanediol[19]. This chemical is a flame retardant, which is the second one in my table here (Slide 6). There were a number of significant cancer findings in this study. The results shown are for male rats; everything I am going to talk about is restricted to male rats, except for 1, 3-butadiene, which is in male mice.

The experimental design in terms of the doses used for 2, 2-bis(bromomethyl)-1,3-propanediol were doses administered in parts per million of feed. There were four exposure categories in the chronic study, control, 2500, 5000 and 10,000 parts per million for 104 weeks. Also, a single stop-exposure group was given 20,000 parts per million of propanediol for 13 weeks. You can scan through these tables and get a feel for the different types of designs that we used in these studies.

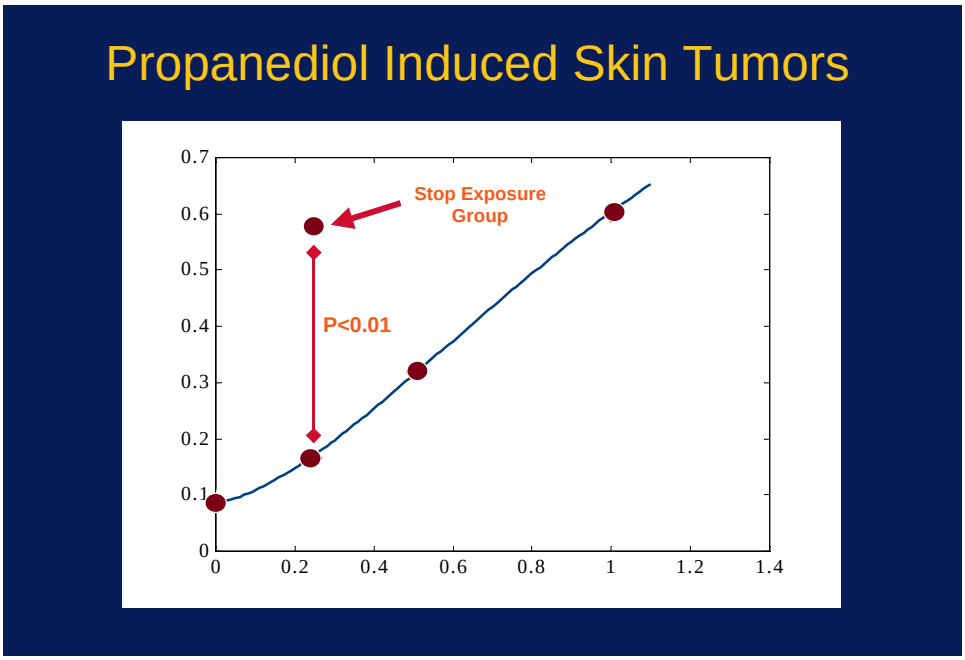
Skin Tumors Following Exposure to
2,2-Bis(bromomethyl)-1,3-propanediol

Dose (ppm)	Dosing Duration (weeks)	Time-Averaged Dose	Animals At Risk	Animals With Tumor	Response (%)
0	104	0	46	4	8.6
2500	104	2500	42	7	16.7
20000	13	2500	36	21	58.3
5000	104	5000	44	14	31.8
10000	104	10000	42	25	59.5

I'm just going to skip through them at this point and go on to the next, and walk you through one of the examples, again, sticking with the 2,2-bis(bromomethyl)-1, 3-propanediol study, which I think reported 18 separate tumor sites. I am just going to pull one of those tumor sites up for you to see what we are talking about and how this is used in the risk assessment.

This particular study has, as I mentioned, five separate groups, one of which is the stop-exposure group which was given 20,000 parts per million. The dosing duration in that group was 13 weeks. If you do a time average on that dose, then instead of it being 20,000 parts per million for 13 weeks, the equivalent dose would be 2500 parts per million for 104 weeks, roughly one-eighth of the original dose. The number of animals at risk is the survival-adjusted number of animals at risk. The numbers with tumors are given, and the percentage responding are in column 6.

What you can see in this table immediately is that the stop-exposure group, which is supposed to be an equivalent dose of 2500 parts per million, clearly does not match the response seen in the group of animals that were actually given 2500 parts per million for the entire length of the study. This group had much higher response than would be expected under chronic exposure at the time-averaged dose.



Now looking at all of them, not just that one, there are three ways in which I can summarize these data and address the question of the importance of stop-exposures, or short-term exposures relative to long-term exposures. Let us start by looking at a statistical test of whether or not the response seen in the stop-exposure groups at the time-averaged dose is significantly different from what would be predicted by fitting a model through the chronic-exposure doses. That is what this picture implies; it is again skin tumors resulting from chronic-exposure doses of 2,2-bis(bromomethyl)-1, 3-propanediol. The smooth curve is a flexible model fit through these data, and as you can see, it almost fits perfectly. This point above the line is the stop-exposure group averaged to lifetime exposure. The distance between the stop-exposure group and the line is what we are looking for in terms of the statistical significance. In this case it is a p-value of 0.01. This type of test can be generalized to more than one stop-exposure group, and that is what I have done in this analysis.

Statistical Significance (p<0.1)

Chemical	Stop larger than Chronic	Stop less than Chronic	Mixed	No Change
Dibromo-anthraquinone		1		4
Propanediol	12			6
Butadiene	5		3	5
Coumarin				1
Dihydro-coumarin				2
Furan	1			

Statistical Significance (p<0.1)

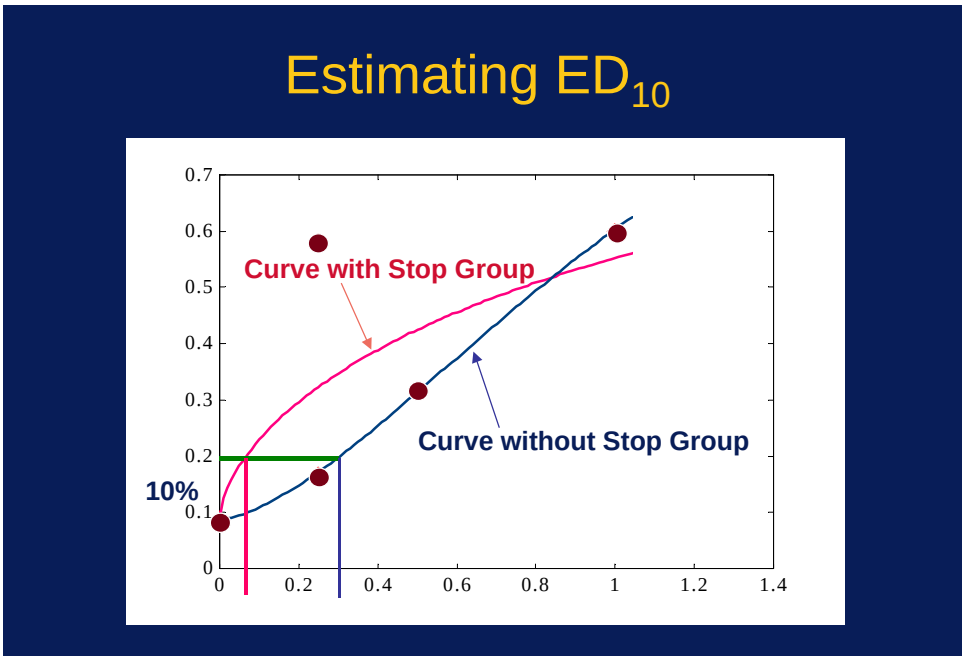
Chemical	Stop larger than Chronic	Stop less than Chronic	Mixed	No Change
Methyleugenol	2			5
o-Nitroanisole	5	2		1
Oxazepam				1
Penta-chlorophenol	2			
Salicylazo-sulfapyridine	1			1

The next two tables describe our findings in this type of analysis, categorizing them into the four obvious categories. I will just go through one to give you some indication of what is in this table. Again we're looking at 2,2-bis(bromomethyl)-1, 3-propanediol. It had 18 significant tumor sites from the study. Of those 18 sites, in 12 of the sites the stop-exposure group was significantly different from the chronic-exposure group prediction (column 2). And in all 12

cases the response seen in the stop-exposure group was much larger, significantly larger ($p<0.10$) than what would have been predicted by the chronic exposure. In six of the cases we could not detect a statistically significant change (column 5). However of those six cases, five of them were above and only one was below. So it was not statistically significant, but it was still above. You can go through all of these and see all of the findings (Slides 12-13). Mixed responses were observed for butadiene, because it had so many stop exposures sometimes one of the points was above, sometimes one of the points was below, but the overall effect was still statistically significant. That is what the "mixed" category (column 4) means, for which butadiene has three tumor sites. The cases in which the stop-exposure response was significantly below that predicted by time-averaging fell into the category labeled "stop less than chronic" (column 3). Adding up the counts from the two tables (Slides 12-13), roughly for 50% of all of the individual tumor sites we looked at, the stop-exposure responses were significantly above the response predicted by the time-averaging dose. In only three of the roughly 60 or so cases were they significantly below. In three of the cases there were mixed responses. And in roughly 50 or 45% of the cases there was no statistical significance between the stop-exposure groups and the chronic groups.

This is a highly significant finding and should not have occurred by chance; you should not have seen 50% of the responses significantly different from what the chronic would present to you. So that was the first way of looking at the data.

The second way of these data is to look at predictions of risk. I am not going to go all the way down into the low-dose range, but I do like to predict somewhere slightly outside the range of the data so I am going to predict the dose that gives a 10 % cancer response above background in these animals for each of these individual compounds with and without including the stop-exposure groups.



That is what this picture shows (Slide 14). Again, we are looking at 2,2-bis(bromomethyl)-1, 3-propanediol-induced skin tumors. The lower curve is the model fit through the data, using only the chronic exposure data. The upper curve is the same model but now fit through all of the data including the time-averaged stop-exposure group. As you can see, this stop-exposure study point is above the other points and it pulls up the curve upward to be able to fit all of this data simultaneously. You get a significant lack of fit, obviously, but you also get a slight change in the curve. The ED_{10} is at 19%, since background response is 10%, that is, a 10% increase over background. The dose that gives you a 10% added response is obtained by going over to this curve and drawing a straight line down to the dose axis. And you see I have shown two of them, the one on the right is for the chronic data alone, and the ED_{10} on the left is for the chronic data and the stop group. As you can see, there is a big difference between these two in terms of the impact it has on the estimate of that ED_{10}.

Changes in ED_{01}

Chemical	Greater than 2-fold decrease	Greater than 2-fold increase	Can't compare	Less than 2-fold change
Dibromo-anthraquinone	1			4
Propanediol	12		2	3
Butadiene	8		2	3
Coumarin	1			
Dihydro-coumarin				2
Furan				1

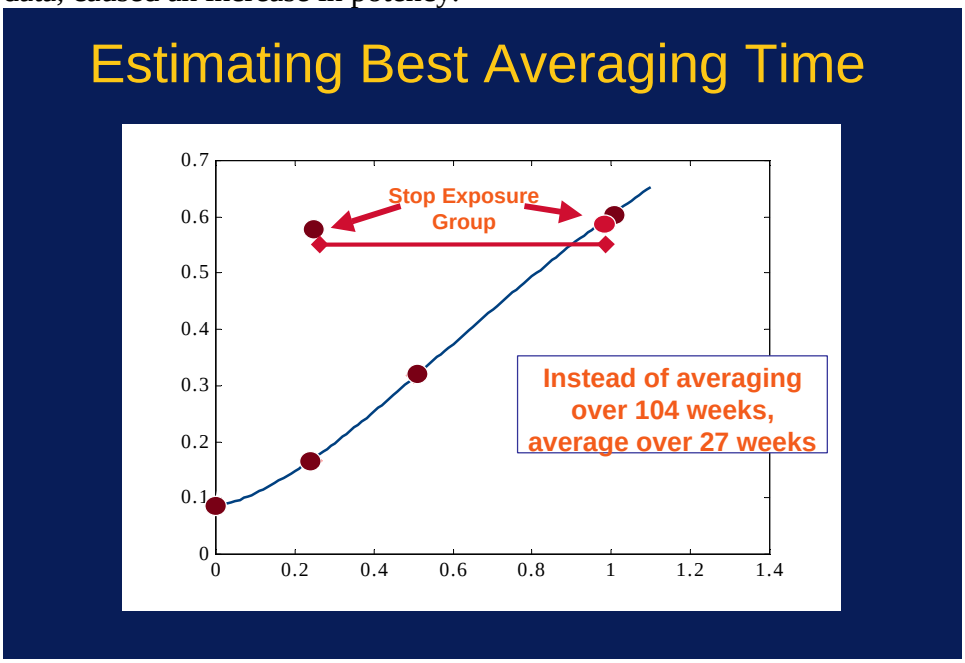
Changes in ED_{01}

Chemical	Greater than 2-fold decrease	Greater than 2-fold increase	Can't compare	Less than 2-fold change
Methyleugenol	1	1		5
o-Nitroanisole	1	1	4	1
Oxazepam				1
Penta-chlorophenol			2	
Salicylazo-sulfapyridine				1

The results for all 12 studies are summarized in this table (Slide 15). Going back to 2,2-bis(bromomethyl)-1, 3-propanediol (listed as propanediol in this table), what I did was break it up into cases where (1) there was a greater than two-fold decrease in the ED_{10} (column 1), (2) where there was greater than twofold increase (column 3), (3) where we could not make a

comparison because of some numerical problems associated with zero tumors in some of the groups (column 4), and (4) less than twofold change in any direction (column 5).

Again, what you find is that the predominant response is in column 2 where you get a greater than twofold increase in the ED_{10}. A decrease in the ED_{10} indicates an increase in potency of the chemical agent. So the time-averaged stop-exposures, when included into the analysis of the data, caused an increase in potency.



Finally, the last question I can ask, or another way to look at this issue is: how much time should I have averaged over? Instead of averaging the dose over 104 weeks, would I have done better if I averaged over 52 weeks, or if I averaged over 75 weeks? Actually, I can calculate the averaging that would have given me perfect agreement between the stop-exposure groups and the model fit through the chronic-exposure data. That is what this graph indicates (Slide 17), again showing the original data points for propanediol-induced skin tumors. The stop exposure group to the left of the graph is the case where I divided it by 104 in order to get the lifetime-averaged dose of 2500 parts per million. However, if I had divided by 27 weeks instead of dividing by 104 weeks, that would have put the dose directly in line with the chronic data as seen to the right of the graph, and the response now perfectly lines up with the model.

Equivalent Averaging Time

- Example
  - » 1000 ppm given for 13 weeks
  - » 52 week equivalent averaging time
  - » all of the following would yield equivalent response
    - – 1000 ppm for 13 weeks averaged over 52 weeks
    - – 250 ppm for 1 year
    - – 250 ppm for 2 years

The general question is what should routinely be used as an averaging time; if there is an obvious trend in the data, what does this mean? One key point I wanted to make is at the bottom of this slide (Slide 18). Suppose I had given a thousand parts per million for 13 weeks but averaged it over 52 weeks instead. That is, suppose my optimum averaging time was 52 weeks, what does that mean in terms of doses? That means that a thousand parts per million for 13 weeks is equivalent to 250 parts per million for one year. But there's something else to be learned here, that is, that I cannot tell between 250 parts per million for one year and 250 parts per million for two years. It is telling me that there may be some time point at which I can stop exposure because the rest of the exposure does not really have much of an impact on the overall

Best Averaging Times

Chemical	52 Weeks or less	52 to 78 Weeks	Longer than 78 Weeks	Median
Methyleugenol	2	1	4	110
o-Nitroanisole	2,1		1,1	36,64
Oxazepam		1		61
Penta-chlorophenol				NA
Salicylazo-sulfapyridine				NA

E)

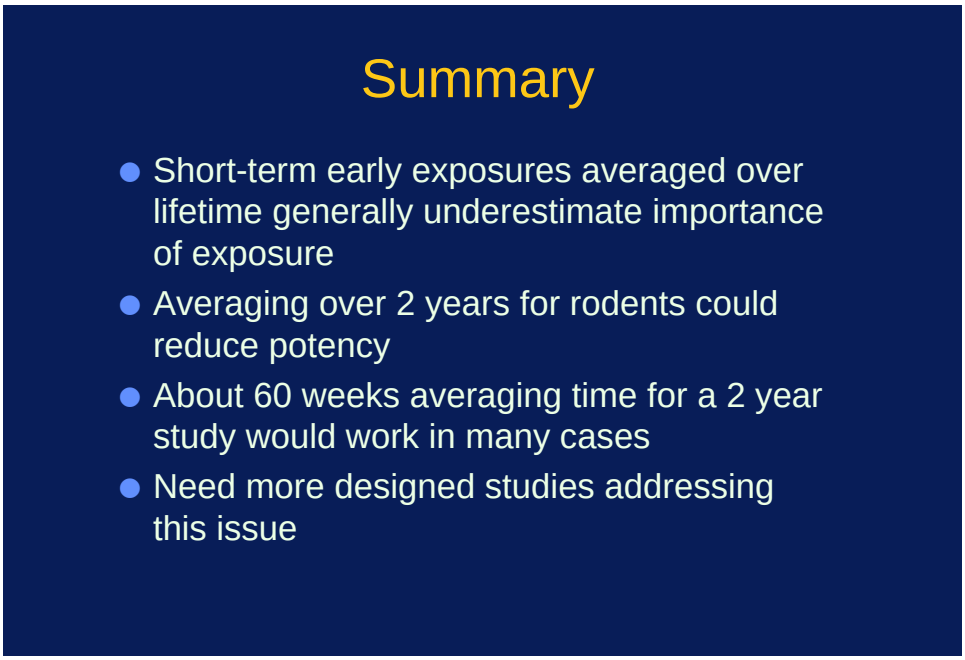
Best Averaging Times

Chemical	52 Weeks or less	52 to 78 weeks	Longer than 78 weeks	Median
Dibromo-anthraquinone	1,0		2,5	88,185
Propanediol	14		2	23
Butadiene	6,8,8,7	2,1,2,2	1,0,1,1	30,52,29, 32
Coumarin		1,1		73,65
Dihydro-coumarin				NA
Furan		1		63

risk that I would see in this group.

Again, dividing the results into categories to summarize the results, and again focusing on propanediol, there were only 16 of the 18 propanediol cases (i.e., tumor types) where I could actually do this. Again, there are problems with this calculation when the tumor response in the stop-exposure group is zero, or when the tumor response in all the chronic groups is zero. Those two cases where calculations were impossible were excluded. What you see is that for 14 of the tumor sites the best averaging time was less than 52 weeks (column 2). For only two of those 16 sites that I could analyze was it longer than 78 weeks (column 4). The median averaging time was 23 weeks (column 6).

If you go through all of these tables and examine all of the information on all of them, you end up with 49 of the 79 cases having averaging times of less than 52 weeks; 12 of them having averaging times between 52 and 78 weeks; and 18 of them having averaging times greater than 78 weeks. If you look across chemicals, there are other comparisons to be made, which I did not summarize. Again, what you see is a propensity for averaging times that should be less than two years in these studies. As an overall average, I would choose something on the order of 60 weeks or so as a general rule.



In summary for this part, short-term early exposures averaged over lifetime generally underestimate the importance of exposure. Averaging over two years for rodents could clearly increase the potency of a chemical agent. About 60 weeks averaging time for a two-year study would probably work in most cases and would not result in responses that are significantly different from the chronic. We need more studies designed to look at the issue of patterns of exposure over age, as well as exposure in chronic studies.

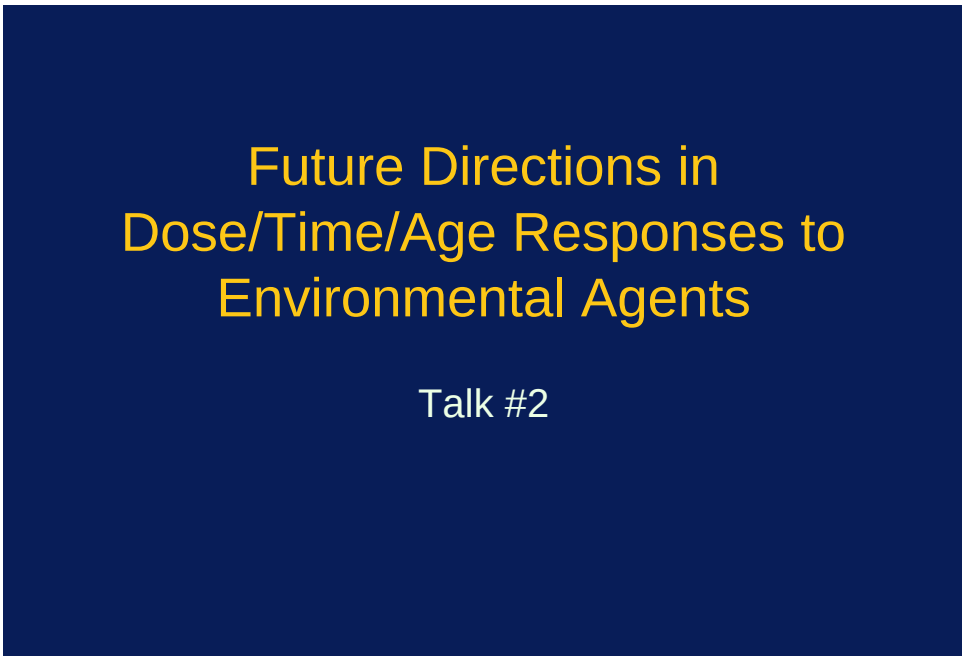
This, and yesterday's presentations, got me to thinking about future directions and where are we going regarding dose, time and age responses for environmental agents. You do not have these slides, but I wanted to make a few points. First, risk assessment is moving clearly away from datasets and more to databases; that is, how do you evaluate and analyze a database for risks, not an individual data set. Second, we are moving away from the simple concepts of hazard assessment and dose response assessment into something a little more complicated. We are asking ourselves four basic questions. Is this a hazard? What is the magnitude of the risk as a function of age, time, dose, *et cetera*? But also we are asking ourselves: What's the shape of the response surface as a function of age, dose, time, *et cetera?* Is there a threshold or not, and,

what's the limit of inference from the data that we have? Where do I begin to extrapolate and where do I have good sound scientific evidence to support my predictions?

Thus, we are really now focused on a much more complex series of questions in looking at the question of is something a hazard and what's the magnitude of the risk. I would argue that this moves us into a requirement for biologically-based modeling and away from some of the empirical work that we have been doing, although there is still a strong role for empirical work.

We have just finished EPA's evaluation of all the dose-response data for dioxins. We looked at every single dataset we could find that indicated something about the dose-response structure for TCDD. We must have looked at somewhere between 600 and a thousand datasets and actually formally analyzed over 200 datasets in this evaluation. These included empirical modeling, mechanistic modeling, all the animal cancer data, non-cancer mechanistic end points, the human data on cancer and heart disease. These analyses were strictly on 2,3,7,8-TCDD.

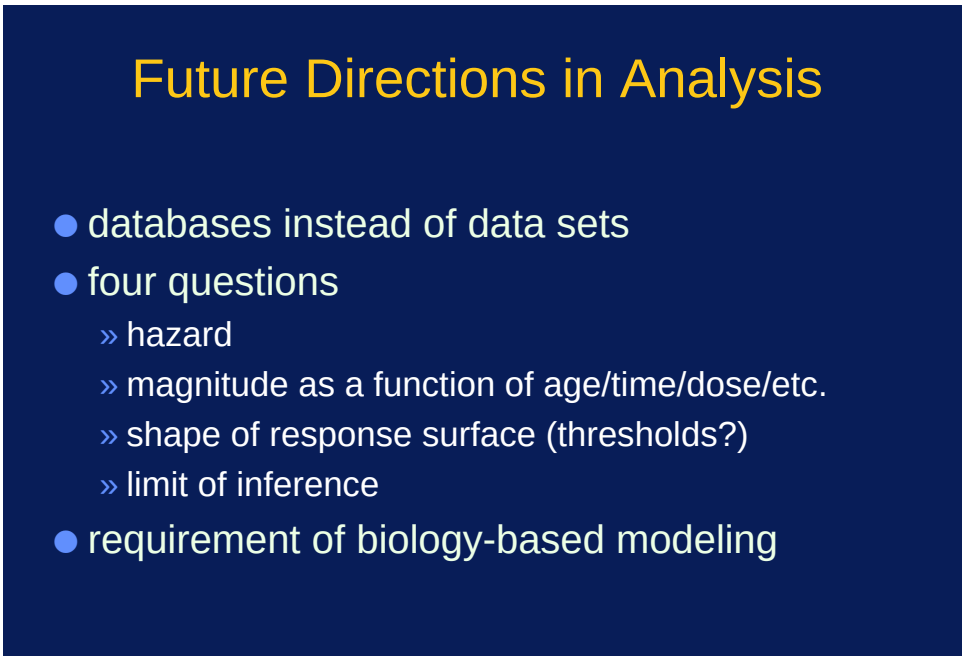
I want to demonstrate for you why I am saying it needs to be a database analysis, not a dataset analysis. I am going to only show you two very simple points from this complicated document.



Yesterday, presenters talked about body burdens and their role in risk. As shown in this picture, these are body burdens associated with a 1% increased risk for a variety of different endpoints from exposure to TCDD. The endpoints are categorized by the type of endpoint rather than by looking at individual data sets. I will read off my slide so you can see them. These are biochemical endpoints, endpoints strictly associated with hepatic function (not hepatic biochemical endpoints, but more frank or functional endpoints in the liver), immune endpoints, retinol, thyroid function and response, and tissue toxicity.

The purpose of this graph is to show that there is a general trend from biochemical endpoints that are very close to the Ah-receptor up towards the more distant end point like tissue toxicity.

These are all based on short-term, multi-dose studies, not the chronic/cancer end points. But you can gain some insight about the question of do I base my risk upon the acutely-toxicity endpoints or biochemical endpoints, and how does that relate to the tissue toxicity.



On the other hand, let us look at single-dose studies. I pulled this out so you can see that they suggest the same sorts of results. The only difference is the previous graph was adults and this graph is both adults and developmental end points. I want to focus on three different groupings: biochemical, and tissue response, and tissue toxicity.

This green line indicates equal ED_{01}s across developmental and adults. You can see the adult biochemical response and the developmental biochemical response are effectively the same. The adult tissue response is slightly higher than biochemical but there is no change in the developmental end points. The adult toxicity end points are much higher. The y-axis represents $\log_{10}$ differences in ED_{01}s, so that adult mean response ranges over three orders of magnitude difference in ED_{01}. Yet for the developmental endpoints, we have a flat line or no change. These data clearly indicate that for TCDD, the developmental response is substantially different than the response seen in adults from single-exposures. We also have graphs like this for chronic-exposure, for human data, *et cetera*. It shows some of the complexity that you can pull out of this information by analyzing the database, not the individual data sets.

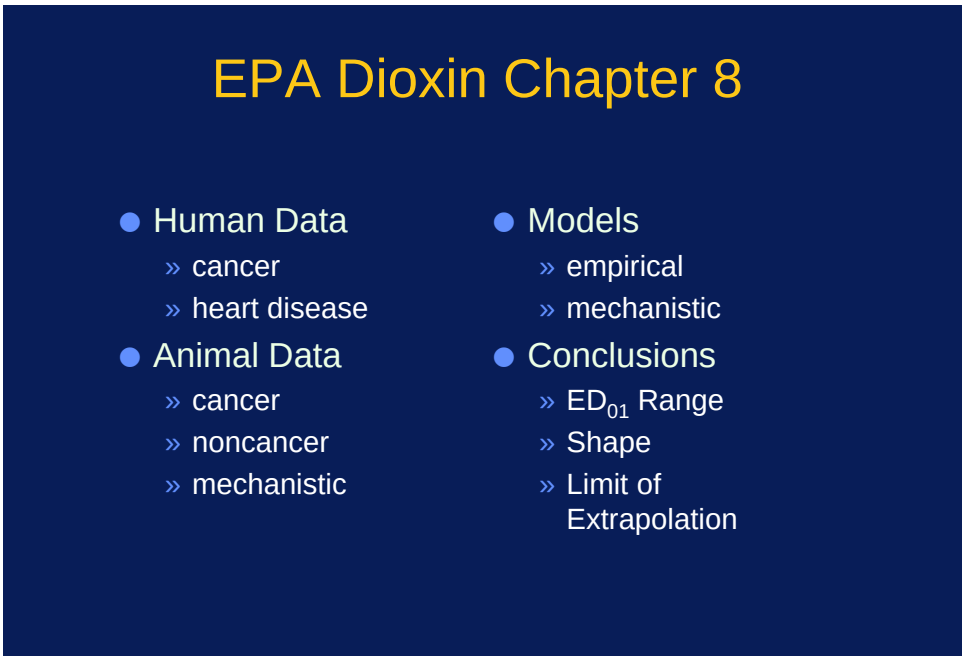
The second point I wanted to make about the future is that technology is going to be a driving force in how we do toxicology in the future. In terms of biology we are going to have more

measures, add more times, add more end points than you ever wanted to look at in your entire life. They are going to be linked to the individual, so we are not going to actually be looking necessarily at just population-based measures. People are going to use gene chips, protein chips, other types of mechanisms which will provide more information on an individual than we will know what to do with.

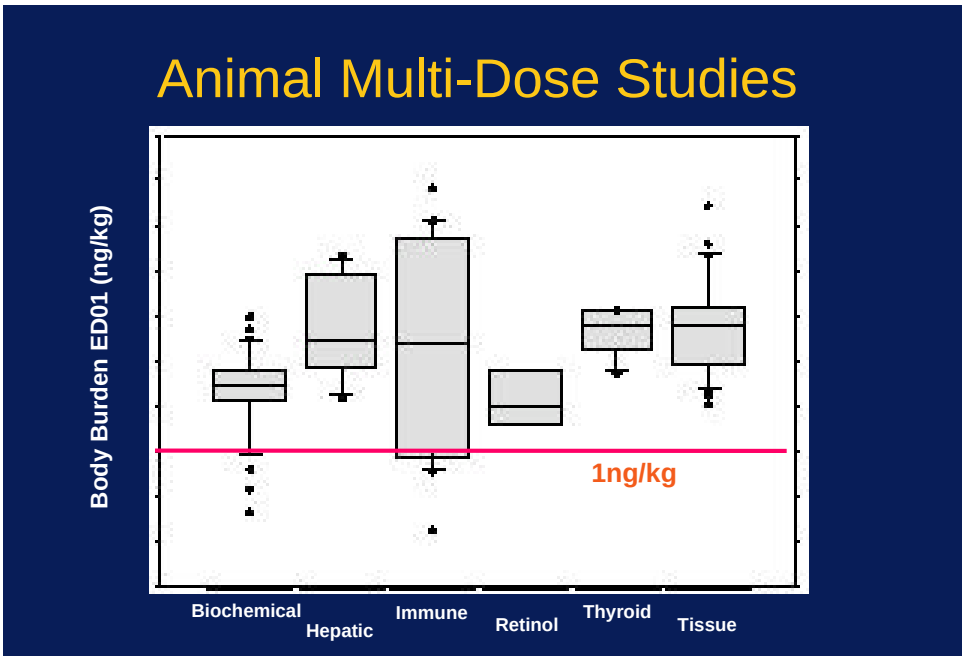
Robotics is going to play a much greater role. The technology used for robotics is improving such that we are going to have much greater throughput. We will not only have more end points but we will also have more replicates of these end points. Thus, we are going to have a lot more information, and there is going to be much greater standardization in how it is done because we are not going to be doing it ourselves, the robotics are going to do a lot of it for us.

Also, the technology of computing is going to substantially change in how we analyze and understand our data. The analysis capacity of modern computers is incredible compared to what it was just five and seven years ago, and it is going to quantum-leap again in the next five years. We are going to be able to conduct analyses we did not even think about in the past. We are going to be able to share information much better than we have in the past.

Tools like artificial intelligence will likely be utilized. I am a big fan of using artificial intelligence in the analysis of data, especially as a great tool for finding data, organizing it into a database for analysis, and presenting it to you in a way that you can then run in an analysis program. So I can see some great movement in those tools.



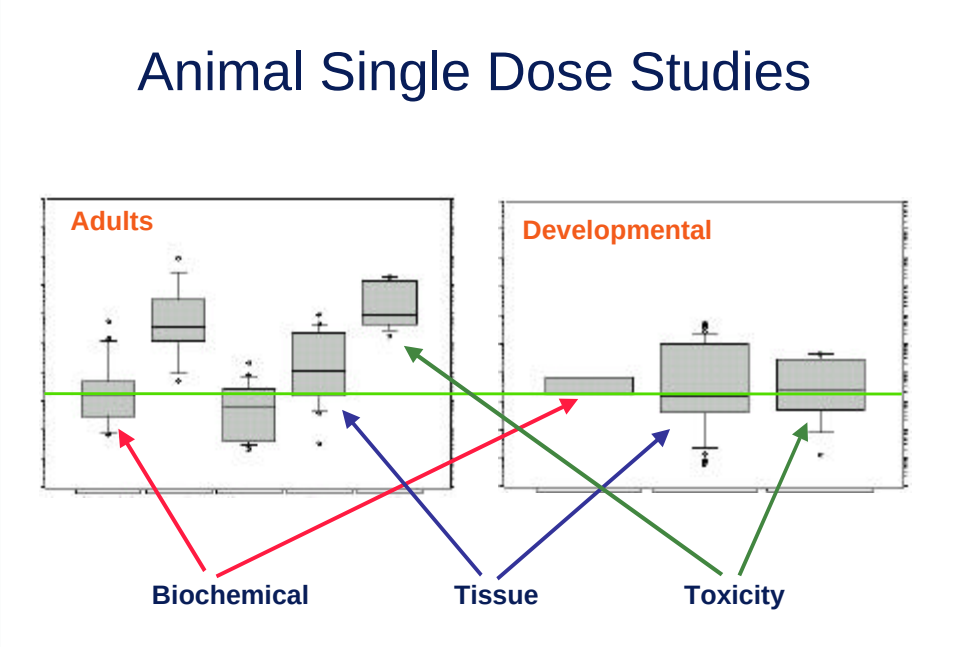
I want to illustrate this, looking at the cDNA microarray technology, where researchers can evaluate changes in numerous biochemical end points of interest. For example, the cDNA chip that is illustrated in slide 24 represents a subset of the genes on the cDNA chip that we currently have at NIEHS, which has 6,000 genes on it. This slide illustrates a subset of the genes that were looked at in one experiment for peroxisome proliferators. Can you imagine looking at 6,000 end points and trying to analyze them simultaneously? The good news is that this is great information. The bad news is, well, "how do we analyze it?" The good news is, we already know how to do it, the bad news is we just do not have the tools to do it yet; we have not developed them.



This is a cell-cycle signal cascade from Kohn (1999)[24] that just appeared. The point is that we know a lot of what happens in some of these signal cascades. In fact in this case not only do we know what happens but Kohn has developed a model that works on the computer pointing out the important pathways, and what signals turn on or off various responses.

If researchers link this model with the chip technologies for proteins and genes, we will have a great tool for looking at chemically-related effects as a function of age, as a function of time since exposure, and as a function of magnitude of exposure. This is the type of tool we are going to need to use in looking at this type of technology, and this is going to need to be linked with the actual overt toxicity that we are going to be trying to find in some of the more mechanistic *in-vivo* studies.

In summary I would say that it is clear that we would have age-period cohort effects, and they play a role in environmental-mediated disease. Technology will likely play a major role in what we are going to achieve in toxicology in the near future. Health risks have to change to take into account this new technology. That means multi-disciplinary, highly-complex, biologically-based methods of analysis are going to be required; they are not going to be the option.



My collaborators on the evaluation of stop-studies are Christine Halmes from TERRA, Steve Roberts and Keith Tolson from the University of Florida, and a number of researchers from NIEHS, and also my brother, Kenneth Portier, from the University of Florida. Thank you very much.

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